Helicobacter pylori and IL-23R gene polymorphism role in degeneration of gastric mucosa

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Running title: Role of IL-23R gene in gastric mucosa

Abstract

Relationship between *H.pylori* (Hp) colonizes and gastric inflammation is widely accepted. Polymorphisms in inflammation related genes such as cytokines and their receptors were thought to partly determine the outcome of Hp infection. Interleukin 23 receptor (IL23R) may relate to degeneration of gastric mucosa. We evaluate association of IL23R +2199 rs10889677 polymorphism and grade of Hp infection with degeneration of gastric mucosa and grade of Hp infection. Biopsies taken from the corpus patients were classified as Hp-infected and Hp-uninfected. The histological severity of Hp infection and degeneration of gastric mucosa were graded from normal to severe. Polymorphism in IL23R was evaluated by PCR-RFLP. AC genotype was related to mild degeneration in Hp-infected subjects (P=0.017). Mild and moderate grades of Hp infection were found related to mild grade of gastric mucosal degeneration (P=0.004 for mild and P=0.037 for moderate grade), sever grade was associated with non-degeneration (P=0.010). We didn't found any association between IL-23R +2199 polymorphism and grades of Hp infection (P>0.05). We concluded that AC genotype of IL-23R polymorphism influences degeneration of gastric mucosa according to presence of Hp and grades of Hp infection.

Keywords: degeneration, IL-23R, polymorphism, Helicobacter pylori, mucosa

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Introduction

Helicobacter.pylori (Hp) is a spiral-shaped gram-negative flagellate bacterium that colonizes the gastric mucosa of approximately 50 % of the world's population [1, 2]. Hp infection induces inflammation in gastric mucosa that involved in chronic gastritis and ulcer [3-5]. It may also lead to precancerous lesions looking like monoclonal lymphocytic proliferation, lymphoid follicle (LF) development and later primary gastric lymphoma (PGL) which develop only in a portion of individual with gastritis because of multifactorial effects of host virulence and bacterial factors that vary among different racial and social groups [6]. Among host factors, several inflammatory proteins including cytokines, growth factors, and chemokines have been known to control adaptive immune response in contrast to Hp infection [7-8]. Firstly, El-Omar was reported an association between gastric cancer risk and interleukin 1 gene cluster polymorphisms [9]. Studies from the western world show roles of anti - and pro -inflammatory cytokine genes such as interleukin (IL)-1β, its receptor antagonist (IL-1RN), IL-10, and tumor necrosis factor (TNF-α) gene polymorphisms affect risk for gastritis [10-11] and GC [12], including its precursors [13-15].

IL-23 is a heterodimer composed of heterodimer of p40/p19 in which p40 is the common subunit shared with IL-12 and p19 is the special subunit with higher affinity to IL-23 receptor (IL-23R) [16]. Recently, an inflammation pathway of IL-23/IL-17 axis reported to play fundamental role in inflammatory and autoimmune diseases [17], such as psoriasis [18], lupus nephritis [19], and intestinal inflammation [20]. There is high level expression of IL-23 in Hp-infected gastric mucosa [21]. IL-23R, as the key component to IL-23 receptor, was shown to play an influential role in the Launching, supporting and accelerating of this IL-23/IL-17 inflammatory signal transduction pathway [22]. In 2006, Duerr et al. indicated the strong relation between Crohn's disease and polymorphisms of the IL-23R gene [23]. Different genotypes of IL-23R gene were evaluated for association with chronic inflammatory disorders [24]. From then, IL23R gene was shown to be the impressionable gene to many other autoimmune/inflammatory diseases. Among the recognized polymorphisms of IL-23R, the functional SNP of +2199A/C (rs10889677) located in the 3´-

untranslated region (UTR) was repeatedly shown to be related to different autoimmune/inflammatory diseases. However, the results are in debate in different groups that have different diseases.

In a study from Hungary, the AA genotype of rs10889677 reported as a risk factor for rheumatoid arthritis [25]. However, another study shows that A allele has a protective role for ankylosing spondylitis [26]. Contradictory, some study indicated that wild type C allele increased the risk to Graves' ophthalmopathy [27] and idiopathic dilated cardiomyopathy[28]. In the present study we therefore aimed to evaluate an association of IL23R +2199A/C polymorphism and grade of Hp infection with degeneration of gastric mucosa, using a case-control approach.

Subjects and methods

438-patients with non-ulcer dyspepsia (NUD) who were undergoing upper gastrointestinal endoscopy were tested for Hp infection using in-house RUT. Hp infected and uninfected patients were determined by the rapid urease test, PCR 16srRNA [26], urea and histological examination of biopsies taken from the corpus. Patients were classified as Hp-infected only if the three tests were positive and Hp-uninfected if the three tests were negative, respectively. Demographic and clinical data were obtained through interview using a standard clinical pro forma. Exclusion criteria included history of gastric neoplasm or surgery, liver disease, and previous treatment with non-steroidal anti-inflammatory drugs, proton pump inhibitors, antibiotics, or bismuth salts. All the study subjects signed informed consents for participation. The Clinical Research Ethics Committee of the Shahrekord University of Medical Sciences approved the study protocol.

Histological examination

Sections of biopsy specimens were embedded 10 % buffered formalin and stained with Hematoxylin and eosin to examine gastritis and with Giemsa to detect Hp. The histological severity of Hp infection and degeneration of gastric mucosa were blindly graded from normal to severe according to the Updated Sydney system on a four-point scale: 0, no; 1, mild; 2, moderate; and 3, severe changes [30].

DNA isolation

Genomic DNA was extracted from biopsies taken from the corpus using Biospin Tissue genomic DNA Extraction Kit (Bio Flux, Japan). All extracted DNA was resuspended in UltraPure RNAse/DNAse-Free Distilled water.

Genotyping for IL23R +2199A/C (rs10889677) polymorphism

Genotyping analysis IL23R genotyping was performed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) as reported by Chen *et al* [31]. Primer sequences for +2199A/C variation of IL23R gene are as follows: sense 5´-AGGGGATTGCTGGGCCATAT-3´, anti-sense 5´-TGTGCCTGTATGTGTGACCA-3´. The PCR amplification was performed in a total volume of 25 µL mixture containing: 100 ng genomic DNA, 1.0 mM of each primer, 200 mM of each dNTP, 2.0 mM of MgCl2 and 1.0 U Taq DNA polymerase and 10 X Taq buffer (Fermentas) using the Biometra Tgradient 96 (Biometra, Germany). PCR conditions were as follows: denaturation at 95 8C for 5 min, followed by 38 cycles of 95 ^{o}C for 30 s, 60 ^{o}C for 45 s, and 72 ^{o}C for 60 s. A final extension was carried out at 72 ^{o}C for 10 min and cooling down to 4 ^{o}C. The PCR products were digested by restriction endonuclease MnLI (Fermentas), according to the manufacturer's instructions, at 37°C overnight and separated by 10% polyacrylamide gel electrophoresis. Gel analysis was performed after staining with ethidium bromide. PCR products were shown to be digested into three types of fragments (Fig. 1). To confirm the genotyping results, selected PCR samples in both groups including samples of each genotype were re-genotyped by other laboratory personnel. There was no difference after genotyping the randomly selected samples.

Statistical analysis

Data were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL). Hardy–Weinberg equilibrium in all subjects was analyzed with the x^2 goodness-of-fit test before the ensuing analyses. The confounding effects of age and gender were adjusted using conditional logistic regression. In addition, Statistical analysis was performed by non-paired T-test depending on the data set. Values of $P<0.05$ were considered as significant.

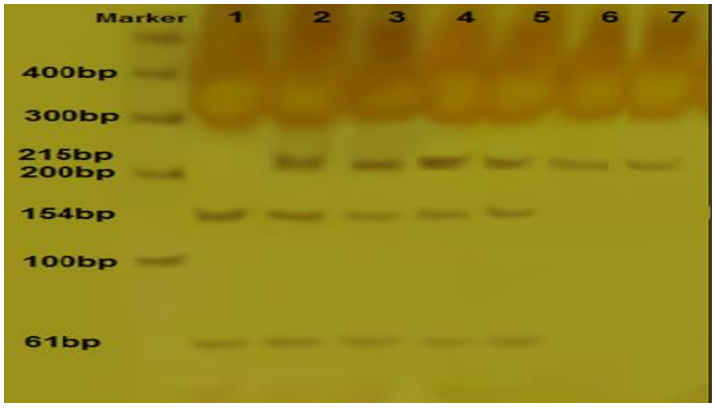

Figure 1.
PCR-RFLP polyacrylamide gel electrophoresis of the IL23R +2199A/C (rs10889677) polymorphism indicating the No.1 (CC = 154, 61 bp), 2, 3, 4, 5 (AC = 215, 154, 61 bp), 6, 7 (AA= 215 bp) genotypes.

Variable	Hp-infected (%)	Hp-uninfected (%)	*P* value
Overall	196(44.7%)	242(55.3%)	
Gender			
Male	79(42.2%)	108(57.8%)	
Female	117(46.6%)	134(53.4%)	0.208
Age			
Mean±SD (year)	47.05 ±17.317	48.29 ±19.493	0.487

Table 1.

Demographic data of study subjects

	*non-degeneration (%)	mild degeneration (%)	moderate degeneration (%)	severe degeneration (%)	*P* value
IL23R +I2199					
AA	36(50.7%)	37(46.8%)	8(27.6%)	9(60.0%)	0.123
AC	23(32.4%)	28(35.4%)	17(58.6%)	2(13.3%)	0.017
CC	13(18.1%)	14(17.7%)	4(13.8%)	4(26.7%)	0.773

* The histopathological parameters were scored as: 0, none; 1, mild; 2, moderate; 3, severe.

Table 2.

Degeneration of gastric mucosa in relation to IL23R +2199 genotypes in Hp-infected subjects

	*non-degeneration (%)	mild degeneration (%)	moderate degeneration (%)	severe degeneration (%)	*P* value
IL23R +I2199					
AA	42(50.6%)	19(46.3%)	26(40.0%)	25(46.3%)	0.648
AC	20(24.4%)	18(43.9%)	23(35.4%)	13(24.1%)	0.131
CC	21(25.6%)	4(9.8%)	16(24.6%)	16(29.6%)	0.082

* The histopathological parameters were scored as: 0, none; 1, mild; 2, moderate; 3, severe.

Table 3.

Degeneration of gastric mucosa in relation to IL23R +2199 genotypes in Hp-uninfected subjects

	*non-degeneration (%)	mild degeneration (%)	moderate degeneration (%)	severe degeneration (%)	*P* value
***Hp grade**					
Mild +	31(44.3%)	45(57.0%)	7(24.1%)	3(20.0%)	0.004
Moderate ++	25(35.7%)	31(39.2%)	15(51.7%)	11(73.3%)	0.037
Severe +++	12(17.4%)	3(3.8%)	7(24.1%)	1(6.7%)	0.010

* The histopathological parameters were scored as: 0, none; 1, mild; 2, moderate; 3, severe.

Table 4.

Hp grades in relation to degeneration of gastric mucosa in Hp-infected subjects

	mild Hp+ *(%)	moderate Hp++ (%)	severe Hp+++ (%)	*P* value
IL23R +I2199				
AA	39(44.8%)	43(51.8%)	8(33.3%)	0.258
AC	33(37.9%)	26(31.3%)	11(45.8%)	0.380
CC	15(17.2%)	14(16.9%)	6(24.0%)	0.699

* The histopathological parameters were scored as: 0, none; 1, mild; 2, moderate; 3, severe.

Table 5.

IL23R +2199 genotypes in relation to Hp grade in Hp-infected subjects.

Results

Demographic and clinical characteristics

Genomic DNA was obtained among the 196 (44.7%) Hp-infected and 242(55.3%) Hp-uninfected gastritis then the DNA all subjects were genotyped. The demographic data of all subjects were demonstrated in Table 1. There was no significant difference between the two groups with respect to the age and gender distribution (P>0.05).

IL23R +2199A/C polymorphism and degeneration of gastric mucosa

In our study population, IL23R +2199A/C (rs10889677) variants (AA, AC, CC) evaluated in Hp-infected and Hp-uninfected population. Genotypes of IL23R+2199A/C were not associated with degeneration of gastric mucosa in Hp-uninfected subjects (P>0.05). In addition, genotypes of AA and CC were not associated to degeneration of gastric mucosa in Hp-infected group (P>0.05) whereas genotype of AC was related to risk for mild degeneration (P=0.017) in these group (Table 3 and 4).

Hp grades infection and degeneration of gastric mucosa

Grade of Hp were comparable among Hp-infected subjects with different grade of Hp

infection (Table 7). Patients with mild (+) and moderate grades (++) of Hp were associated with mild degeneration of gastric mucosa (P=0.004 for mild and P= 0.037 for moderate grade) whereas patients with severe grade (+++) was related to non-degeneration (P = 0.010).

IL23R +2199A/C polymorphism and grades of Hp infection

As shown in table 5 below, probable role of IL23R +2199A/C variants (AA, AC, CC) in related to grades of Hp infection evaluated in Hp-infected group. Not all genotypes of IL23R + 2199A/C were associated with different grades of HP infection in this group (P>0.05).

Discussion

In the present study we found that IL23R+2199AC genotype increases susceptibility to mild degeneration of gastric mucosa in patients infected with Hp but we don't observed this effect for the same genotype in Hp-uninfected patients that may indicate Hp infection is associated with AC genotype outcome in pathway of IL-23/IL-17 axis that result in degeneration of gastric mucosa. In addition, we found that variants of IL23R gene, IL23R +2199AA, IL23R +2199 CC, were not associated with degeneration of gastric mucosa in non-infected and patients infected with Hp.

These findings suggest these IL23R polymorphism my independent of the presence and/or absence of Hp has no effect on degeneration. Whereas, one study regardless of Hp role suggest IL23R +2199CC, genotype significantly decreased gastric cancer risk and some of IL23R+2199A/C genotypes associated with increased risk of certain subtypes of gastric cancer, but not with all of them [31]. This may indicate that the effect of IL23R polymorphism on inflammatory processes varied with inflammatory response steps. This result is consistent with the different mechanisms of inflammation so that in precancerous and degeneration stages some of cytokines are dominant and have specific role in start of inflammation process but as stage progress, another cytokines participate therefore, we observe many cytokines affection and decreased effectiveness of special polymorphisms in the latter stages.

As there is no enough biological report that revealed the function of IL23R +2199 polymorphism, especially in precancerous, it is difficult to fully elucidate this phenomenon about our study. A study reported a higher levels of IL-23 in Hp-infected patients (including DU and AS groups) than in the Hp negative control group [32]. In addition, studies revealed that the inflamed gastric mucosa of Hp-positive patients could secrete IL-23 may corroborate our findings [33]. However, another study reported no significant difference in mucosal IL-17 and IL-23 mRNA expression between Hp-infected and a non-infected patient [34] that may

contradicts with our study. Gastric mucosa of patients with both duodenal and gastric ulcers was equally potent for secretion of IL-23 compared with patients with chronic active gastritis with no signs of peptic ulcer disease. The release of IL-23 was greater by Hp-infected gastric mucosa than by gastric mucosa not infected by Hp mainly for patients with chronic gastritis and only after stimulation with LPS. Similar findings have been published elsewhere [21, 34]. Nevertheless, LPS of Hp has also been described to behave in a different manner [35]. A study reported many Hp factors have significant association with duodenal ulcers [36] for example cagA positivity correlated with gastritis that show cagA virulence factor may have initiated role in precancerous stages. The results of one study also showed that bacterial factors might act as inducers of IL-23 [32].

Our findings showed mild and moderate grades of Hp were related to susceptibility to mild degeneration of gastric mucosa we observed sever grade of Hp was associated with non-degeneration. We suggest mild and moderate grade of Hp have direct and more effect on gastric mucosa as we can see the effect of Hp in degeneration but as grade of Hp increases, in sever grade of Hp, immune system responses to Hp infection and prevents a possible role of HP in degeneration. Persistent colonization depends on the ability to respond to changing environmental conditions and circumvent host defense mechanisms initiated during infection [37]. We don't found any possible role of IL-23R+2199A/C polymorphism in capacities to a certain degree of Hp infection, that suggest genotypes of IL-23R+2199. My don't have the role in susceptibility to certain grade of Hp solitary and /or my exist set of factors in related to grade of Hp infection, then we unable to observed probable effect of IL-23R+2199A/C polymorphism in this phenomena. In this study in particular, we have demonstrated that AC genotype of IL-23R play a role in mild degeneration of gastric mucosa. Whether IL23R is a dependent or independent mediator in the pathogenesis of gastric mucosa or not cannot be excluded with safety from the presented findings. Further investigation is necessary to elucidate fully the exact role of IL-23R polymorphism in the pathogenesis of gastric mucosa. Our results highlight the importance of Hp severity infection in explaining degeneration outcomes after infection with Hp. However, the importance of host genetic factors rather than Hp virulence in explaining variations in outcomes after infection in different Asian countries has been reported [38].

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